

Linkage Analysis of the X-linked Green Tail and Blue Tail Color Genes in the Guppy, *Poecilia reticulata*

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ABSTRACT—Linkage between the X-linked green tail gene (*Grt*) and blue tail gene (*Blt*) determining tail color in the Green Snakeskin and Blue Snakeskin varieties of the guppy (*Poecilia reticulata*), respectively, was determined from segregation data of F₂ progeny from single-pair reciprocal crosses between these two varieties. The *Grt* and *Blt* genes are both dominant and X-linked but they are non-allelic. The average map distance between them was found to be 19.4 map units.

INTRODUCTION

Fancy strains of guppies have been commercially cultured in Singapore since the early 1950's. Over the years through intensive selection of new color mutants and hybridization between strains by guppy hobbyists and commercial breeders, the number of fancy varieties have been steadily increasing. To date about 30 different varieties are reared on monoculture farms in Singapore [1]. Previous studies showed that the green tail color of the Green Snakeskin variety (GSS) was mediated by an X-linked dominant gene, *Grt* [2]. Genetic studies on the blue tail coloration of the Blue Tail and Blue Snakeskin (BSS) varieties showed that it was determined by an X-linked dominant gene (*Blt*) [3-6]. The iridescent snakeskin body and tail patterns, characteristic of males of the BSS and GSS varieties were confirmed by Phang *et al.* to be determined by two closely linked genes (*Ssb* and *Sst*) on the Y-chromosome [2, 6]. However, studies have not been carried out to determine whether the X-linked *Blt* and *Grt* genes are allelic. Thus the aim of this paper was to test for allelism and linkage relationships between these two X-linked tail color genes.

MATERIALS AND METHODS

Source of the fish

Two- to three-week old fry of the Blue Snakeskin and Green Snakeskin varieties were obtained from a fish farm in Singapore. Virgin females were required for the crosses since fertilization in the guppy is internal and females can retain sperm for several months after a single insemination. The fry were raised in 33 liter clear plastic tanks (20 fish/tank) in the aquarium area of the Department of Zoology, National University of Singapore, at temperatures of 26-28°C. Sexual differentiation takes place at 4-6 weeks of age under laboratory conditions. The young fish were checked daily for developing males and these when spotted were immediately removed and raised separately from females.

Description of the varieties

Adult BSS males are characterized by silvery snakeskin-like pattern on the body, and dark navy-blue colored tails (Fig. 1A). Genetic studies on the BSS variety have shown that males carry three sex-linked genes which determine the color phenotype; the X-linked blue tail gene (*Blt*) and the Y-linked snakeskin body (*Ssb*) and snakeskin tail (*Sst*) genes [6]. GSS males carry the the X-linked *Grt* gene which gives the greenish-yellow tail color



FIG. 1A. A pair of Green Snakeskin guppies. Female (above) and male (below)
 Fig. 1B. A pair of Blue Snakeskin guppies. Female (right) and male (left)

and the Y-linked *Ssb* and *Sst* genes responsible for the snakeskin body pattern and the black snakeskin-like reticulations on the tail, respectively (Fig. 1B). Both BSS and GSS females have the normal wild-type body coloration and do not have the snakeskin patterns. BSS females have partial expression of the navy-blue tail color, while GSS females have slight green and grey spotting on the

tail.

Reciprocal crosses

Testing of allelism between the X-linked *Blr* and *Grt* genes present in the BSS and GSS varieties, respectively, was conducted by performing reciprocal crosses between the two varieties. Single-pairs were set up using 3-month old sexually

mature virgin fish. The pairs were kept in eight liter breeding tanks. The following notations were used for the crosses:

Cross 1: BSS male \times GSS female

Cross 2: GSS male \times BSS female

F₁ broods were usually produced 4–6 weeks after mating. The segregation of color phenotypes among the F₁ progeny was recorded. Phenotypic proportions among the F₁ progeny were subjected to Chi-square tests. F₂ broods were obtained from single-pair matings between full-sib F₁ progeny. F₁ females that were not used for breeding and all F₂ females were treated with the androgen, methyl testosterone, to manifest sex-limited color genes carried by them [7, 8].

RESULTS

Cross 1 (Blue Snakeskin male \times Green Snakeskin female)

A total of 119 male and 93 female F₁ progeny were obtained from 12 matings of this cross (Table 1). All F₁ males had the GSS phenotype with the distinct snakeskin body and greenish-yellow tail with black reticulations. Thus the F₁ males inher-

ited the X-linked green tail gene (*Grt*) from the GSS female parent and the Y-linked snakeskin body and tail genes (*Ssb* and *Sst*) from the BSS male parent. The caudal fin of the F₁ females had a tinge of dark blue. After treatment of F₁ females with androgen, blue and greenish-yellow color manifested prominently on the tails showing that they inherited the X-linked blue tail (*Blt*) and green tail (*Grt*) genes from their male and female parents, respectively. The F₁ females were classified as having the blue tail, green tail phenotype (BGT). The Chi-square test showed that the observed male and female F₁ data conformed to the expected 1:1 ratio ($\chi^2=3.19$, $P>.05$).

Table 2 shows the proposed genetic model for the segregation of the two X-linked gene loci (*Blt* and *Grt*) for Cross 1 and the resulting F₁ and F₂ progeny. Both F₁ and F₂ data supported this model and showed the X-linked *Blt* and *Grt* genes were non-allelic. Crossing-over between the *Blt* and *Grt* genes in the heterozygous F₁ females resulted in four color phenotypes among the F₂ male progeny (Table 2). The two parental phenotypes among the F₂ males were BSS and GSS. The two recombinant F₂ male phenotypes were; Blue Green Snakeskin (BGSS) which could be

TABLE 1. Segregation of color phenotypes among the F₁ and F₂ progeny of single-pair matings of Cross 1 (Blue Snakeskin male \times Green Snakeskin female) and Cross 2 (Green Snakeskin male \times Blue Snakeskin female)

Cross (Cross No.)	Gen.	No. of matings	No. and phenotypes of Progeny	
			Males	Females
BBS \times GSS (Cross 1)	F ₁	12	119 GSS	93 BGT
	F ₂	15	45 GSS	65 GT
			49 BSS	60 BGT
			*13 BGSS	
			*15 WTSS	
GSS \times BSS (Cross 2)	F ₁	11	98 BSS	83 BGT
	F ₂	17	77 BSS	97 BT
			84 GSS	81 BGT
			*13 BGSS	
			*17 WTSS	

BSS : Blue Snakeskin phenotype

GSS : Green Snakeskin phenotype

BGT : Blue, Green Tail phenotype

GT : Green Tail phenotype

*BGSS : Blue, Green Snakeskin phenotype (recombinant type)

*WTSS : Wild-type Snakeskin phenotype (recombinant type)

TABLE 2. Proposed genetic model to show segregation and crossing-over of tail color genes (*Blt* and *Grt*) in the cross between a Blue Snakeskin male and Green Snakeskin female (Cross 1)

CROSS 1	
P	<div> BLUE SNAKESKIN MALE $X_{Blt+,Grt+}$ $Y_{Ssb,Sst}$ </div> × <div> GREEN SNAKESKIN FEMALE $X_{Blt+,Grt}$ $X_{Blt+,Grt}$ </div>
F ₁	<div> $X_{Blt+,Grt}$ $Y_{Ssb,Sst}$ Green Snakeskin males </div> ↓ <div> $X_{Blt+,Grt}$ $X_{Blt+,Grt+}$ Blue/Green Tail females </div>
F ₂ NON-CROSSEOVERS	<div> $X_{Blt+,Grt}$ $Y_{Ssb,Sst}$ Green Snakeskin males </div> ↓ <div> $X_{Blt+,Grt}$ $X_{Blt+,Grt}$ Green Tail females </div> <div> $X_{Blt,Grt+}$ $Y_{Ssb,Sst}$ Blue Snakeskin males </div> ↓ <div> $X_{Blt+,Grt}$ $X_{Blt,Grt+}$ Blue/Green Tail females </div>
F ₂ CROSSEOVERS	<div> $X_{Blt,Grt}$ $Y_{Ssb,Sst}$ Blue/Green Snakeskin males </div> ↓ <div> $X_{Blt,Grt}$ $X_{Blt+,Grt}$ *Blue/Green Tail females </div> <div> $X_{Blt+,Grt+}$ $Y_{Ssb,Sst}$ Wild-Type Snakeskin males </div> ↓ <div> $X_{Blt+,Grt+}$ $X_{Blt+,Grt}$ *Green Tail females </div>

* Crossover F₂ females cannot be distinguished from non-crossover F₂ females

recognized by the snakeskin body and greenish-yellow tail with large dark blue patches and black reticulations, and; Wild-type Snakeskin (WTSS) which characteristically had the iridescent snakeskin body pattern and silvery reticulations on the hyaline tail (Fig. 2A and 2B). The total number of F₂ non-crossovers was 94 and that of crossovers was 28 (Table 1). The recombination frequency between the *Blt* and *Grt* genes estimated from the percentage of crossover males of the total F₂ males ($28/122 \times 100\%$) was 23.0%.

Table 2 showed that the crossover value could not be obtained from the F₂ female data since the recombinant phenotypes were identical to the parental ones.

Cross 2 (Green Snakeskin male × Blue Snakeskin female)

Twelve matings of this cross gave a total of 98 F₁ males with BSS phenotype and 83 F₁ females with blue and green tail phenotype (BGT) (Table 1). The data conformed to the expected sex ratio of 1 male:1 female ($\chi^2=1.24$, $P>.05$). The F₁ males inherited the X-linked *Blt* gene from the BSS female parent and the Y-linked *Ssb* and *Sst* genes from the GSS male parent. The F₁ females inherited the X-linked *Blt* and *Grt* genes from the BSS female and GSS male parent, respectively.

There were four color phenotypes among the F₂ male progeny due to crossing-over and recombination (Table 3). Among the non-crossovers were 77 BSS males and 84 GSS males (Table 1). The



FIG. 2A. A crossover F_2 male with snakeskin body and greenish-blue snakeskin tail (Blue, Green Snakeskin-BGSS).



FIG. 2B. A crossover F_2 male with snakeskin body and hyaline tail with snakeskin pattern (Wild-type Snakeskin-WTSS) (above). A F_2 female with wild-type phenotype (below).

crossover F_2 males consisted of 13 BGSS and 17 WTSS (Fig. 2A and 2B). The recombination frequency between the *Blt* and *Grt* genes calculated as the percentage of crossover males to the total number of F_2 males ($30/191 \times 100$), was found to be 15.7%.

As in Cross 1, Table 3 showed that the F_2 female data could not give any estimate of recombination frequency since non-crossovers and crossovers had identical phenotypes.

DISCUSSION

Data on crossing-over have been used in the mapping of sex chromosomes in the guppy [5, 6, 9–11]. Two estimates of recombination frequency between the X-linked *Blt* and *Grt* genes were obtained from the F_2 data of reciprocal crosses between the BSS and GSS varieties. From Cross 1 the crossover value was 23.0% while that from Cross 2 was 15.7% giving an average map distance of 19.4 units between the X-linked *Blt* and *Grt* genes, showing that these two genes are not closely linked. However, the discrepancy between the

TABLE 3. Proposed genetic model to show segregation and crossing over of tail color genes in the cross between a Green Snakeskin male and Blue Snakeskin female (Cross 2)

CROSS 2					
P	<div> GREEN SNAKESKIN MALE × BLUE SNAKESKIN FEMALE </div>				
	<div> $X_{Blr^+,Grt} \ Y_{Ssb,Sst}$ $X_{Blr,Grt^+} \ X_{Blr,Grt^+}$ </div>				
F ₁	<div> ↓ </div> <table> <tr> <td> $X_{Blr,Grt^+} \ Y_{Ssb,Sst}$ Blue Snakeskin males </td><td> $X_{Blr,Grt^+} \ X_{Blr^+,Grt}$ Blue/Green Tail females </td></tr> </table>	$X_{Blr,Grt^+} \ Y_{Ssb,Sst}$ Blue Snakeskin males	$X_{Blr,Grt^+} \ X_{Blr^+,Grt}$ Blue/Green Tail females		
$X_{Blr,Grt^+} \ Y_{Ssb,Sst}$ Blue Snakeskin males	$X_{Blr,Grt^+} \ X_{Blr^+,Grt}$ Blue/Green Tail females				
F ₂ NON-CROSSOVERS	<div> ↓ </div> <table> <tr> <td> $X_{Blr,Grt^+} \ Y_{Ssb,Sst}$ Blue Snakeskin males </td><td> $X_{Blr,Grt^+} \ X_{Blr,Grt^+}$ Blue Tail females </td></tr> <tr> <td> $X_{Blr^+,Grt} \ Y_{Ssb,Sst}$ Green Snakeskin males </td><td> $X_{Blr^+,Grt} \ X_{Blr,Grt^+}$ Blue/Green Tail females </td></tr> </table>	$X_{Blr,Grt^+} \ Y_{Ssb,Sst}$ Blue Snakeskin males	$X_{Blr,Grt^+} \ X_{Blr,Grt^+}$ Blue Tail females	$X_{Blr^+,Grt} \ Y_{Ssb,Sst}$ Green Snakeskin males	$X_{Blr^+,Grt} \ X_{Blr,Grt^+}$ Blue/Green Tail females
$X_{Blr,Grt^+} \ Y_{Ssb,Sst}$ Blue Snakeskin males	$X_{Blr,Grt^+} \ X_{Blr,Grt^+}$ Blue Tail females				
$X_{Blr^+,Grt} \ Y_{Ssb,Sst}$ Green Snakeskin males	$X_{Blr^+,Grt} \ X_{Blr,Grt^+}$ Blue/Green Tail females				
F ₂ CROSSOVERS	<table> <tr> <td> $X_{Blr,Grt} \ Y_{Ssb,Sst}$ Blue/Green Snakeskin males </td><td> $X_{Blr,Grt} \ X_{Blr,Grt^+}$ *Blue/Green Tail females </td></tr> <tr> <td> $X_{Blr^+,Grt^+} \ Y_{Ssb,Sst}$ Wild-Type Snakeskin males </td><td> $X_{Blr^+,Grt^+} \ X_{Blr,Grt^+}$ *Blue Tail females </td></tr> </table>	$X_{Blr,Grt} \ Y_{Ssb,Sst}$ Blue/Green Snakeskin males	$X_{Blr,Grt} \ X_{Blr,Grt^+}$ *Blue/Green Tail females	$X_{Blr^+,Grt^+} \ Y_{Ssb,Sst}$ Wild-Type Snakeskin males	$X_{Blr^+,Grt^+} \ X_{Blr,Grt^+}$ *Blue Tail females
$X_{Blr,Grt} \ Y_{Ssb,Sst}$ Blue/Green Snakeskin males	$X_{Blr,Grt} \ X_{Blr,Grt^+}$ *Blue/Green Tail females				
$X_{Blr^+,Grt^+} \ Y_{Ssb,Sst}$ Wild-Type Snakeskin males	$X_{Blr^+,Grt^+} \ X_{Blr,Grt^+}$ *Blue Tail females				

* Crossover F₂ females cannot be distinguished from non-crossover F₂ females

two crossover values meant that more estimates have to be obtained before firmer conclusions could be drawn.

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